

## **Secretion Verification**

**Goal:** Verify the activity of a signal peptide in *B. subtilis* by measuring the fluorescence of mCherry in extracellular medium.

### **Strains for this experiment:**

- *B.subtilis* with signal peptide + mCherry
- *B.subtilis* with mCherry
- *B.subtilis* with empty pDR111 (no mCherry)

### **Protocol:**

1. Transfer from glycerol stock to 5 ml LB+spectinomycin and incubate at 37°C overnight (16 hr).
2. Dilute the *Bacillus* 1/100 (50ml LB + 50ul spectinomycin) in 250 ml Erlenmeyer and check O.D. at 600 nm.
3. Check O.D. every 1 hr to verify growth rate until the solution gets to O.D.=0.6.
4. Transfer to 50 ml falcon and centrifuge for 5 min at 5000 g. Resuspend the pellet in BA (bioassay) medium.
5. Add 0.1 mM IPTG (5 µl).
6. Samples will be taken at time 0 (at 0.6 O.D.) and every 1 hr thereafter.
7. Transfer a 1 ml sample from each Erlenmeyer into Eppendorfs:
  1. Transfer 200 µl to a 96 well plate for measuring O.D.600 nm in duplicate.
  2. Centrifuged the remaining sample at max speed for 2 minutes.
8. Transfer 200 µl of the supernatant in duplicate into 96 well plate.
9. Check O.D. at 600 nm and fluorescence at excitation= 587 nm, emission= 610 nm in plate reader.